The antineoplastic effect of the novel compound LQB-118 in multicellular tumor spheroids of colorectal cancer cells

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Background: Colorectal cancer (CRC) is the third most common cancer in men and second in women worldwide. The CRC is characterized by diverse molecular alterations. The great majority patients exhibit hyper-activation of the Wnt pathway, what makes it an attractive target for therapeutic intervention. Despite the development of new strategies, such as introduction of new molecular-targeted pharmacologic agents, the success rate of treatment is still poor. The standard treatment for CRC is surgery resection followed by adjuvant chemotherapy using 5-Fluorouracil (5-FU). However, 5-FU shows high toxicity for cardiomyocytes and is associated with aggressive myelosuppression. To overcome this problem, new therapeutic strategies are needed to improve the success rate and reduce toxicity in CRC treatment. Our previous data shows that the novel compound LQB-118, a pterocarpanoquinone derived from the lapachol molecule, is very promising in the treatment of CRC. LQB-118 induces higher percentage of cell death in CRC cell lines than 5-FU and causes low toxicity towards organs of immune system *in vivo*.

Aims: To evaluate the antitumor effect of LQB-118 on CRC cell lines grown as monolayer and spheroid and test its potential as a modulator of the Wnt pathway.

Results and Discussion: LQB-118 reduced cell viability of both HCT-116 and HT-29 CRC cell lines in a concentration-dependent fashion. However, HCT-116 showed a higher IC50 when compared to HT-29, indicating to be more resistant to the novel compound. 5-FU reduced cell viability in a time-dependent fashion and equally in both cell lines. Comparing LQB-118 to 5-FU, our data shows that LQB-118 reduces cell viability in concentrations subtoxic to human cells, while the concentrations used for 5-FU are associated to cytotoxicity in patients. AnnexinV/PI staining revealed that the compound LQB-118 induces cell death in concentrations higher than 2,5 µM in 24 hours in both cell lines. Both 5-FU and LQB-118 reduced the formation of colonies in the cell lines treated in all concentrations for 24 hours without cell cycle arrest. Since 3D cell culture is a more reliable and relevant tool for drug screening, this methodology was tested in CRC cell lines incubated with LQB-118 and 5-FU. Analysis of spheroids volume and viability after treatment with LQB-118 revealed that the compound reduces the growth rate and induces cell death. Additionally, LQB-118 reduced cell migration while 5-FU exhibited no effect in this 3D model. To evaluate the possible modulation of Wnt pathway, the CRC cell lines were transduced with a gene reporter responsive to Wnt activity. We are still setting the experimental conditions to evaluate the impact of the compound in this pathway.

Conclusions: By inducing cell death and inhibiting cell migration in a more effective way than the standard treatment (5-FU), LQB-118 is a promising compound to overcome 5-FU limited effects and probably inhibiting metastases.

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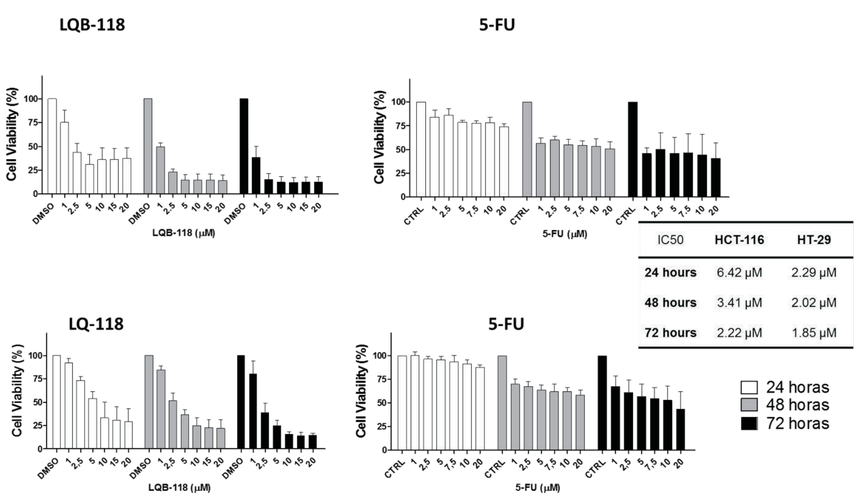


Figure 1. HCT-116 and HT-29 cell viability after treatment with 5-FU or LQB-118. HCT-116 and HT-29 cells were treated with the compounds for 24, 48 and 72 hours and cell viability was acessed via MTT assay. Cells treated with DMSO were used as control. Mean and standard deviation of 3 independent experiments IC 50 calculated using Graphpad Prism V4.0

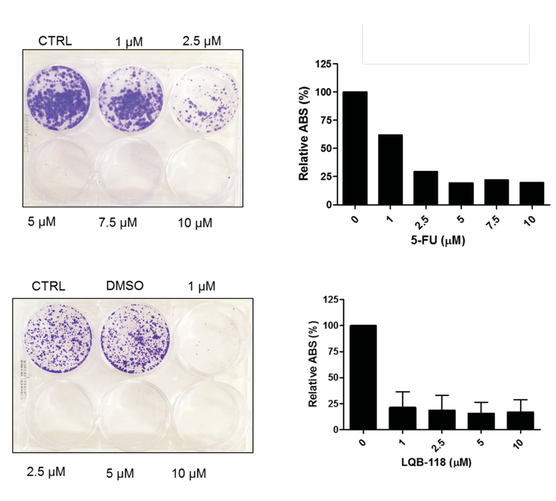
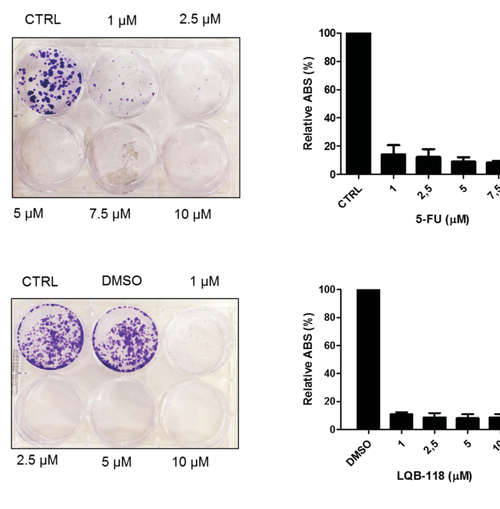


Figure 2. Clonogenic assay with HCT 116 cells treated with LQB-118 or 5-FU. After adhered, the colonies stained with violet crystal were dissolved in acetic acid and absorbance was obtained at 595 nm. **A:** Absorbance relative to colonies treated with 5-FU. **B:** Absorbance relative to colonies treated with LQB-118 The results represent mean and SD of 2 and 3 independent experiments for 5-FU and LQB-118, respectively.



Clonogenic assay with HT-29 cells treated with LQB-118 or 5-FU. After adhered, the colonies stained with violet crystal were dissolved in acetic acid and absorbance was obtained at 595 nm. **A:** Absorbance relative to colonies treated with 5-FU. **B:** Absorbance relative to colonies treated with LQB-118 The results represent mean and SD of 2 and 3 independent experiments for 5-FU and LQB-118, respectively.

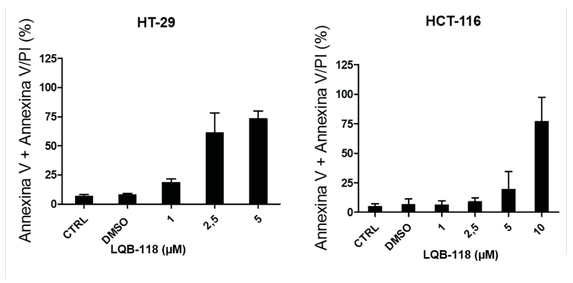


Figure 3. Evaluation of cell death promoted by LQB-118 in CRC HCT 116 cells. After treatment with diferent concentrations of LQB-118 for 24 24 hours, cells were incubated with Annexin/PI and results acessed by flow cytometry. Mean and SD of three independent experiments

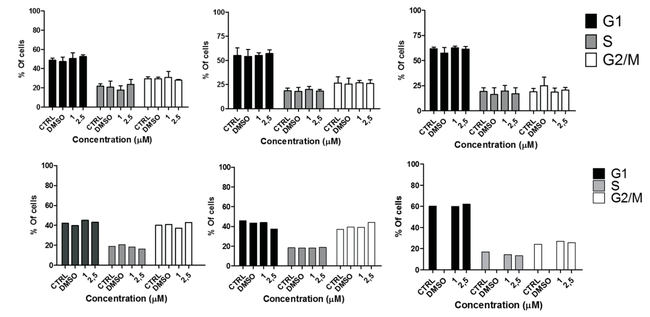


Figure 4. Cell cycle distribution after treatment with LQB-118. HCT-116 and HT-29 cells were treated in monolayer with the compound for 24, 48 and 72 hours and cell cycle distribution was evaluated by DNA content. Mean and sd of 3 experiments for HCT 116 and one experiment for HT-29

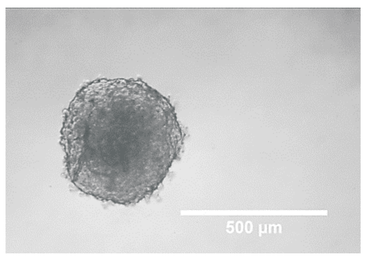


Figure 5. Representative figure of HCT-116 spheroid. After 4 days in the incubator, spheroids have approximately 500 µm of diameter.

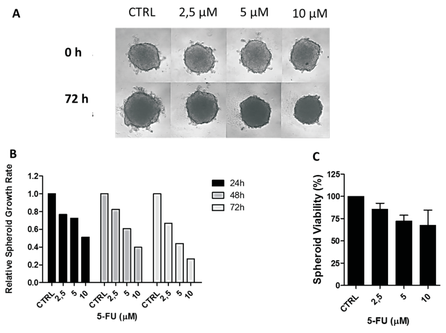


Figure 6. Spheroid growth and viability of HCT-116 spheroids treated with 5-FU. A: Representative figure of spheroid growth after treatment with 5-FU for 72h **B:** Relative spheroid growth kinectics after treatment with. The growth rate of each concentration was analyzed using the volume. Mean of 2 independent experiments. **C:** Cell viability of spheroids treated with 5-FU for 72 hours using the APH assay. Mean and SD of 3 independent experiments

Figure 7. Spheroid growth and viability of HCT-116 spheroids treated with LQB-118. A: Relative spheroid growth kinectics after treatment with LQB-118. The growth rate of each concentration was analyzed using the volume. Mean and SD 3 independent experiments **B:** Cell viability of spheroids treated with 5-FU for 72 hours using the APH assay. Mean and SD of 3 independent experiments **C:** Representative figure of spheroid growth after treatment with LQB-118 for 72h **D:** Full spheroid detachment after 72 hours of treatment with LQB-118

Figure 8. 3D HCT-116 spheroid migration after treatment with LQB-118 or 5-FU. Spheroids were treated with differents concentrations of the novel compound LQB-118 or 5-FU. Images representative of 2 experiments

Figure 9. Experimental procedure of wnt activity analysis using cell lines reporter

Figure 10: Enrichment of mCherry+ cells. A: HCT-116 and HT-29 cell lines were transduced using lentivirus with the plasmid 7TGC **(B)**. For population enrichment, a Cell Sorter was used (FacsMelody BD)

Figure 11: Wnt activation by inhibition of GSK3 using LiCl: The cell lines HCT-116 + 7TGC and HT-29 + 7TGC were treated with 20 mmol of LiCl for 24 hours to activate the canonical wnt pathway

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA